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Surveillance of population dynamics and breeding habitat diversity of *Anopheles subpictus* in different areas of Odisha, East Central India

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Abstract

Odisha state is highly endemic for malaria since long. Among different malaria vectors, *Anopheles subpictus* is known to be one of the most prevalent species that breeds in a variety of fresh as well as saline water habitats. The study of the distribution of mosquitoes, both in natural and anthropologically disturbed areas, is of considerable importance due to their role in pathogen transmission. The main objective of the current study was to detect the breeding habitat diversity of *An. subpictus.* Different larval habitats for Anopheles mosquitoes were surveyed every month in the year 2016. The anopheline larvae were collected using the standard dipping method. The specimens were identified using morphological keys. The result showed indoor/outdoor breeding ratio was 1.16 for *An. subpictus*. The most productive breeding site for *An. subpictus* was earthen pot and cement tank. The Container Index (CI) was found more in indoor and House Index (HI) was found more in outdoor studies respectively. It was also noticed that the prevalence of *An. subpictus* was more in July to September and January to February, which might be because of variations in rainfall due to low pressure, especially during January and February. The current study provides a better understanding of the characteristics of various aquatic habitats and breeding preferences by mosquitoes which can be useful for improving larval source management operations for the control of mosquito vectors.

Keywords: *Anopheles subpictus,* Breeding Habitat Diversity, Container Index, House Index, Population Dynamics

Introduction

Malaria is one of the major communicable diseases causing high mortality and morbidity in India. In 2018, the National Vector Borne Disease Control Programme (NVBDCP) estimated that approximately 500,000 people suffered from malaria, and less than 100 persons died (NVBDCP, 2018). India is the main contributor to malaria in South-East Asia. In India, *Anopheles fluviatilis* and *Anopheles culicifacies* are two important primary vectors for malaria. *Anopheles subpictus* has been reported as a vector in some parts of India (Paniker *et al*., 1981; Kulkarni, 1987; Chatterjee and Chandra, 2000). It was also reported as a predominant vector species for malaria in the state of Odisha (Dash *et al*., 2000). Odisha is a highly malaria-endemic state as it has favourable ecological and geographical conditions and huge areas of forest with tribal settlements (Pradhan *et al.*, 2019). Nearly 46 million population at risk of Malaria infection in Odisha (Pradhan *et al*., 2019).

The Indian Council of Medical Research has launched the 'Malaria Elimination Research Alliance (MERA) India', a conglomeration of partners working on malaria control in order to prioritise, plan and scale up research to eliminate the disease from India by 2030.

Several strategies have been implemented for Malaria control. Entomological surveillance was considered a major tool under the Malaria control programme. Mosquito larval habitat ecology is important in determining larval densities, the relative importance of breeding habitats, and species assemblage as well as designing mosquito control programs (Simsek, 2004). The breeding habitat is crucial for mosquito population dynamics (Reuban, 1978). Therefore, the identification of breeding sites appears to be an easier means to check the mosquito population dynamics. Breeding sites and

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containers in and around human habitation are probably the most important factors facilitating the breeding of mosquito vectors. The present study was undertaken to determine the breeding preferences of *An. subpictus* mosquitoes by conducting a larval survey in 6 different localities of the Ganjam district of Odisha state, India. The larval survey was carried out in both outdoor as well as indoor containers and the Breeding Preference Ratio (BPR) was calculated.

Materials and Methods

Selection of Study Site and Sampling

In the present study, the outdoor survey was made from fixed localities across the three selected towns such as Aska (19° 36' 22.0536" N; 84° 40' 20.9604" E), Buguda (19° 48' 33.7716" N; 84° 47' 30.8796" E), Bhanjanagar (19° 56' 10.8924" N; 84° 34' 43.4784" E) and three rural areas like Bhetanai (19° 41' 2.6268" N; 84° 41' 22.5456" E), Ballipadar (19° 44' 11.526" N; 84° 42' 23.1696" E) and Baunsalundi (19° 55' 28.812" N; 84° 34' 15.3048" E) of Ganjam district of Odisha state to understand the types of breeding sites and breeding preference of certain wild mosquito species (Figure 1).

Figure 1. Map showing (circle marks) study areas.

Table 1. Month-wise containers screened for *Anopheles subpictus* in Buguda

Outdoor study							
Sl No	**Month**	**No. of House Visited**	**No. of Positive case**	**No. of Container screened**	**No. of positive container**	**HI%**	**CI%**
1	January	20	12	200	26	60	13
2	February	20	10	200	24	50	12
3	March	20	11	200	21	55	10.5
4	April	20	04	200	07	20	3.5
5	May	20	05	200	06	25	3.0
6	June	20	09	200	21	45	10.5
7	July	20	13	200	32	65	16
8	August	20	11	200	31	55	15.5
9	September	20	08	200	19	40	9.5
10	October	20	08	200	16	40	8.0
11	November	20	06	200	11	30	5.5
12	December	20	05	200	09	25	4.5
Indoor study							
1	January	20	06	100	11	30	11
2	February	20	11	100	31	55	31
3	March	20	06	100	17	30	17
4	April	20	03	100	05	15	5.0

5	May	20	02	100	06	10	6.0
6	June	20	04	100	06	20	6.0
7	July	20	13	100	14	65	14
8	August	20	09	100	28	45	28
9	September	20	03	100	19	15	19
10	October	20	08	100	27	40	27
11	November	20	06	100	13	30	13
12	December	20	03	100	07	15	7.0

Table 2. Month-wise containers screened for *Anopheles subpictus* in Ballipadar

Outdoor study							
Sl No	**Month**	**No. of House Visited**	**No. of Positive case**	**No. of Container screened**	**No. of positive container**	**HI%**	**CI%**
1	January	20	03	200	12	15	6.0
2	February	20	09	200	29	45	14.5
3	March	20	08	200	26	40	13
4	April	20	02	200	07	10	3.5
5	May	20	02	200	08	10	4.0
6	June	20	06	200	17	30	8.5
7	July	20	09	200	33	45	16.5
8	August	20	11	200	36	55	18
9	September	20	03	200	13	15	6.5
10	October	20	09	200	19	45	9.5
11	November	20	03	200	05	15	2.5
12	December	20	05	200	08	2.5	4.0
Indoor study							
1	January	20	02	100	06	10	6.0
2	February	20	09	100	24	45	24
3	March	20	07	100	09	35	9.0
4	April	20	03	100	09	15	9.0
5	May	20	02	100	05	10	5.0
6	June	20	07	100	18	35	18
7	July	20	09	100	38	45	38
8	August	20	12	100	40	60	40
9	September	20	04	100	11	20	11
10	October	20	07	100	16	35	16
11	November	20	04	100	13	20	13
12	December	20	03	100	13	15	13

Table 3. Month-wise containers screened for *Anopheles subpictus* in Aska

Outdoor study							
Sl No	**Month**	**No. of House Visited**	**No. of Positive case**	**No. of Container screened**	**No. of positive container**	**HI%**	**CI%**
1	January	20	07	200	13	35	6.5
2	February	20	08	200	14	40	7.0
3	March	20	06	200	12	30	6.0
4	April	20	05	200	11	25	5.5
5	May	20	02	200	06	10	3.0
6	June	20	11	200	29	55	14.5
7	July	20	13	200	42	65	21.0
8	August	20	12	200	36	60	18.0
9	September	20	11	200	31	55	15.5
10	October	20	06	200	14	30	7.0
11	November	20	03	200	07	15	3.5
12	December	20	03	200	05	15	2.5
Indoor study							
1	January	20	04	100	18	20	18
2	February	20	06	100	12	30	12
3	March	20	03	100	07	15	7.0
4	April	20	03	100	06	15	6.0
5	May	20	06	100	08	30	8.0
6	June	20	09	100	13	45	13
7	July	20	14	100	29	70	29
8	August	20	11	100	27	55	27
9	September	20	09	100	20	45	20
10	October	20	11	100	19	55	19
11	November	20	06	100	11	30	11
12	December	20	04	100	10	20	10

Table 4. Month-wise containers screened for *Anopheles subpictus* in Bhetanai

Outdoor study							
Sl No	**Month**	**No. of House Visited**	**No. of Positive case**	**No. of Container screened**	**No. of positive container**	**HI%**	**CI%**
1	January	20	03	200	10	15	5.0
2	February	20	06	200	18	30	9.0
3	March	20	05	200	21	25	11.5
4	April	20	03	200	07	15	3.5
5	May	20	02	200	06	10	3.0
6	June	20	09	200	23	45	11.5
7	July	20	13	200	41	65	20.5

8	August	20	12	200	39	60	19.5
9	September	20	09	200	29	45	14.5
10	October	20	05	200	14	25	7.0
11	November	20	05	200	13	25	6.5
12	December	20	03	200	08	15	4.0
Indoor study							
1	January	20	06	100	18	30	18
2	February	20	07	100	21	35	21
3	March	20	04	100	13	20	13
4	April	20	04	100	16	20	16
5	May	20	02	100	06	10	06
6	June	20	11	100	19	55	19
7	July	20	11	100	42	55	42
8	August	20	09	100	28	45	18
9	September	20	05	100	14	25	14
10	October	20	06	100	17	30	17
11	November	20	05	100	14	25	14
12	December	20	02	100	07	10	07

Table 5. Month-wise containers screened for *Anopheles subpictus* in Bhanjanagar

Outdoor study							
Sl No	**Month**	**No. of House Visited**	**No. of Positive case**	**No. of Container screened**	**No. of positive container**	**HI%**	**CI%**
1	January	20	09	200	24	45	12
2	February	20	06	200	19	30	9.5
3	March	20	04	200	24	20	7.0
4	April	20	04	200	09	20	4.5
5	May	20	03	200	07	15	3.5
6	June	20	07	200	13	35	6.5
7	July	20	12	200	39	60	19.5
8	August	20	11	200	32	55	16
9	September	20	11	200	29	55	14.5
10	October	20	09	200	17	45	8.5
11	November	20	05	200	11	25	5.5
12	December	20	02	200	05	10	2.5
Indoor study							
1	January	20	05	100	12	25	12
2	February	20	08	100	17	40	17
3	March	20	06	100	13	30	13
4	April	20	04	100	12	20	12
5	May	20	03	100	09	15	09
6	June	20	09	100	18	45	18

7	July	20	11	100	26	55	26
8	August	20	10	100	29	50	29
9	September	20	11	100	22	55	22
10	October	20	09	100	19	45	19
11	November	20	05	100	12	25	12
12	December	20	04	100	04	20	04

Table 6. Month-wise containers screened for *Anopheles subpictus* in Baunsalundi

Outdoor study							
Sl No	**Month**	**No. of House Visited**	**No. of Positive case**	**No. of Container screened**	**No. of positive container**	**HI%**	**CI%**
1	January	20	08	200	23	40	11.5
2	February	20	06	200	18	30	9.0
3	March	20	07	200	13	35	6.5
4	April	20	04	200	07	20	3.5
5	May	20	02	200	04	10	2.0
6	June	20	06	200	12	30	6.0
7	July	20	13	200	42	65	21.0
8	August	20	11	200	26	55	13.0
9	September	20	12	200	27	60	13.5
10	October	20	07	200	21	35	11.5
11	November	20	05	200	11	25	5.5
12	December	20	03	200	10	15	5.0
Indoor study							
1	January	20	05	100	15	25	15
2	February	20	06	100	19	30	19
3	March	20	04	100	11	20	11
4	April	20	04	100	09	20	9.0
5	May	20	03	100	07	15	7.0
6	June	20	08	100	24	40	24
7	July	20	11	100	38	55	38
8	August	20	10	100	26	50	26
9	September	20	12	100	32	60	32
10	October	20	07	100	24	35	24
11	November	20	04	100	11	20	11
12	December	20	04	100	09	20	9.0

Mosquito Collection and Identification

Immature stages of mosquitoes were collected during the year 2016 from different breeding sites of the selected study area at monthly intervals using standard sampling methods (Tables 1-6). After collection, larvae were reared and identified using identification keys. *Anopheles subpictus* was abundantly found in all most all the selected study areas.

Entomological indices

Entomological indices, namely, BPR (Breeding Preference Ratio), CI (Container Index) and HI (House Index) were computed from the recorded data collected during this work. To calculate these indices, the following formulae were used.

$$\text{Container Index } (\text{CI}) = \frac{\text{Number of positive containers}}{\text{Number of containers inspected}} \times 100$$

$$\text{House Index } (\text{CI}) = \frac{\text{Number of houses infested}}{\text{Number of houses inspected}} \times 100$$

Anopheles Larval Surveillance

All potential breeding habitats were identified in all 6 localities through a preliminary survey conducted for a period of one month prior to the research study and certain fixed and temporary breeding places were identified for the larval survey. Larval collections were made randomly from indoor sites (earthen pot, cement tank, plastic drum, flower pot, and plastic bucket) and outdoor sites (earthen pot, cement tank, old vehicles, discarded tyre, plant axil, coconut shell, bamboo clump, plastic container, metal drum and plant pot). The details of the number and type of habitats surveyed and mosquito species recovered were recorded. The immature stages were collected with the help of a glass dropper and transferred to the laboratory in plastic containers. Larger water containers were sampled as per the protocol (Eshita and Kurihara, 1978; Wongkoon *et al.*, 2005), in brief, by dipping a fish net in the water starting at the top and continuing to the bottom in swirling motion, sampling all edges of the containers.

Results

Assessment of HI and CI of Selected Study Area

Container bioassay was carried out in six selected study areas to understand the breeding preference of *An. subpictus*. A total 21,600 number of containers were screened both in outdoor and indoor containers, out of which 2,542 (11.7%) containers were found positive for larvae of *An. subpictus* (1,317 out of 14,400 and 1,225 out of 7,200 in outdoor and indoor containers). The HI and CI data of selected areas are presented in Figures 2 and 3.

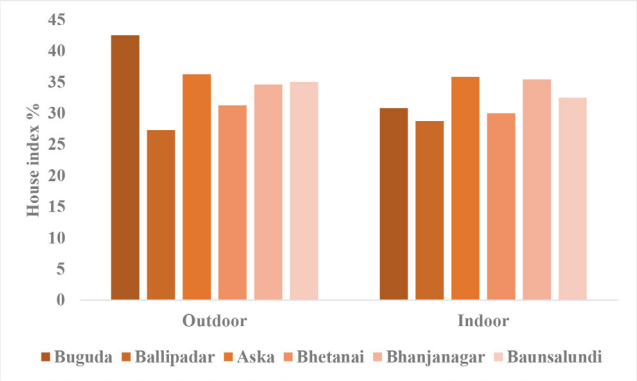

Figure 2. House Index of *Anopheles subpictus* of different localities under study. (Buguda, Ballipadar, Aska, Bhetanai, Bhanjanagar and Baunsalundi).

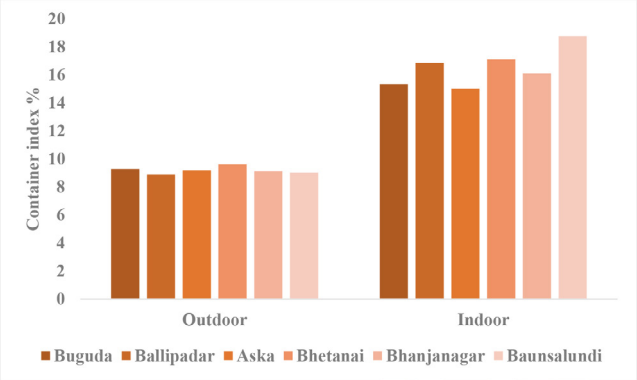

Figure 3. Container Index of *Anopheles subpictus* of different localities under study. (Buguda, Ballipadar, Aska, Bhetanai, Bhanjanagar and Baunsalundi)

Analysis of Breeding Preference Ratio of Selected Study Area

For Breeding Preference Ratio (BPR), 10 varieties of outdoor containers and 5 varieties of indoor containers were selected. The number of containers examined (both outdoor and indoor) was arbitrarily set to 240. Based on the percentage of containers positive for *An. subpictus*,

the X% (calculated by dividing total no. of individual container screened with total no. of containers screened and multiplied by 100), Y% (calculated by dividing total no. of individual container positive cases with total no. of containers screened and multiplied by 100) and BPR (by dividing Y% with X%) values were calculated. BPR for outdoor containers was found maximum for cement tanks and earthen pots but no larvae were found in the bamboo clump in almost all the study areas. Similarly, the BPR for indoor containers was maximum for earthen

Table 7. The breeding preference for different types of container/sites (indoors and outdoors) in *Anopheles subpictus* mosquitoes at Buguda, Odisha, India

SL NO	Outdoor Container	Examined	X%	+ve Cases (%)	Y%	BPR
1	Earthen pot	240	10	38 (15.83)	17.04	1.70
2	Cement tank	240	10	37 (15.41)	16.59	1.65
3	Old vehicles	240	10	19 (7.91)	8.52	0.85
4	Discarded tyre	240	10	20 (8.33)	8.96	0.89
5	Plant axil	240	10	15 (6.25)	6.72	0.67
6	Coconut shell	240	10	24 (10)	10.76	1.07
7	Bamboo clump	240	10	0	0	0
8	Plastic container	240	10	32 (13.33)	14.34	1.43
9	Metal drum	240	10	29 (12.08)	13	1.3
10	Plant pot	240	10	09 (3.75)	4.03	0.40
	TOTAL	2400		223 (9.29)		
	Indoor Containers					
1	Earthen pot	240	20	33 (13.75)	17.93	0.89
2	Cement tank	240	20	44 (18.33)	23.91	1.19
3	Plastic drum	240	20	43 (17.91)	23.36	1.16
4	Flower pot	240	20	26 (10.83)	14.13	0.70
5	Plastic bucket	240	20	38 (15.83)	20.65	1.03
	TOTAL	1200		184 (15.33)		

Table 8. The breeding preference for different types of container/sites (indoors and outdoors) in *Anopheles subpictus* mosquitoes at Ballipadar, Odisha, India

Sl No	Outdoor Container	Examined	X%	+ve Cases (%)	Y%	BPR
1	Earthen pot	240	10	41 (17.08)	19.24	1.92
2	Cement tank	240	10	32 (13.33)	15.02	1.5
3	Old vehicles	240	10	21 (8.75)	9.85	0.98
4	Discarded tyre	240	10	23 (9.58)	10.79	1.07
5	Plant axil	240	10	08 (3.33)	3.75	0.37
6	Coconut shell	240	10	27 (11.25)	12.67	1.26
7	Bamboo clump	240	10	0	0	0
8	Plastic container	240	10	30 (12.5)	14.08	1.4
9	Metal drum	240	10	31 (12.91)	14.55	1.45

10	Plant pot	240	10	0	0	0
	TOTAL	2400		213 (8.87)		
	Indoor Containers					
1	Earthen pot	240	20	52 (21.66)	25.74	1.28
2	Cement tank	240	20	44 (18.33)	21.78	1.08
3	Plastic drum	240	20	38 (15.83)	18.81	0.94
4	Flower pot	240	20	21 (8.75)	10.39	0.51
5	Plastic bucket	240	20	47 (19.58)	23.26	1.16
	TOTAL	1200		202 (16.83)		

Table 9. The breeding preference for different types of container/sites (indoors and outdoors) in *Anopheles subpictus* mosquitoes at Aska, Odisha, India

Sl No	Outdoor Container	Exam-ined	X%	+ve Cases (%)	Y%	BPR
1	Earthen pot	240	10	38 (15.83)	17.27	1.72
2	Cement tank	240	10	31 (12.91)	14.09	1.40
3	Old vehicles	240	10	17 (7.08)	7.72	0.77
4	Discarded tyre	240	10	24 (10)	10.90	1.09
5	Plant axil	240	10	19 (7.91)	8.63	0.86
6	Coconut shell	240	10	23 (9.58)	10.45	1.04
7	Bamboo clump	240	10	0	0	0
8	Plastic container	240	10	29 (12.08)	13.18	1.31
9	Metal drum	240	10	26 (10.83)	11.81	1.18
10	Plant pot	240	10	13 (5.41)	5.90	0.59
	TOTAL	2400		220 (9.16)		
	Indoor Containers					
1	Earthen pot	240	20	48 (20)	26.66	1.33
2	Cement tank	240	20	39 (16.25)	21.66	1.08
3	Plastic drum	240	20	36 (15)	20	1.0
4	Flower pot	240	20	19 (7.91)	10.55	0.52
5	Plastic bucket	240	20	38 (15.83)	21.11	1.05
	TOTAL	1200		180 (15)		

Table 10. The breeding preference for different types of container/sites (indoors and outdoors) in *Anopheles subpictus* mosquitoes at Bhetanai, Odisha, India

Sl No	Outdoor Container	Examined	X%	+ve Cases (%)	Y%	BPR
1	Earthen pot	240	10	39 (16.25)	17.03	1.70
2	Cement tank	240	10	48 (20)	20.96	2.09
3	Old vehicles	240	10	27 (11.25)	11.79	1.17
4	Discarded tyre	240	10	25 (10.41)	10.91	1.09
5	Plant axil	240	10	15 (6.25)	6.55	0.65

6	Coconut shell	240	10	18 (7.5)	7.86	0.78
7	Bamboo clump	240	10	0	0	0
8	Plastic container	240	10	28 (11.66)	12.22	1.22
9	Metal drum	240	10	13 (5.41)	5.67	0.56
10	Plant pot	240	10	16 (6.66)	6.98	0.69
	TOTAL	2400		229 (9.54)		
	Indoor Containers					
1	Earthen pot	240	20	57 (23.75)	26.51	1.32
2	Cement tank	240	20	52 (21.66)	24.18	1.20
3	Plastic drum	240	20	40 (16.66)	18.60	0.93
4	Flower pot	240	20	28 (11.66)	13.02	0.65
5	Plastic bucket	240	20	38 (15.83)	17.67	0.88
	TOTAL	1200		215 (17.91)		

Table 11. The breeding preference for different types of container/sites (indoors and outdoors) in *Anopheles subpictus* mosquitoes at Bhanjanagar, Odisha, India

Sl No	Outdoor Container	Examined	X%	+ve Cases (%)	Y%	BPR
1	Earthen pot	240	10	28 (11.66)	12.84	1.28
2	Cement tank	240	10	36 (15)	16.51	1.65
3	Old vehicles	240	10	26 (10.83)	11.92	1.19
4	Discarded tyre	240	10	21 (8.75)	9.63	0.96
5	Plant axil	240	10	10 (4.16)	4.58	0.45
6	Coconut shell	240	10	27 (11.25)	12.38	1.23
7	Bamboo clump	240	10	05 (2.08)	2.29	0.22
8	Plastic container	240	10	31 (12.91)	14.22	1.42
9	Metal drum	240	10	26 (10.83)	11.92	1.19
10	Plant pot	240	10	08 (3.33)	3.66	0.36
	TOTAL	2400		218 (9.08)		
	Indoor Containers					
1	Earthen pot	240	20	59 (24.58)	26.94	1.34
2	Cement tank	240	20	49 (20.41)	22.37	1.11
3	Plastic drum	240	20	42 (17.5)	19.17	0.95
4	Flower pot	240	20	25 (10.41)	11.41	0.57
5	Plastic bucket	240	20	44 (18.33)	20.09	1.0
	TOTAL	1200		219 (18.25)		

Table 12. The breeding preference for different types of container/sites in *Anopheles subpictus* mosquitoes at Baunsalundi, Odisha, India

Sl No	Outdoor Container	Examined	X%	+ve Cases (%)	Y%	BPR
1	Earthen pot	240	10	20 (8.3)	9.17	0.91
2	Cement tank	240	10	55 (22.9)	25.22	2.52
3	Old vehicles	240	10	34 (14.1)	15.59	1.55
4	Discarded tyre	240	10	22 (9.1)	10.09	1.0
5	Plant axil	240	10	02 (0.8)	0.91	0.09
6	Coconut shell	240	10	18 (7.5)	8.25	0.82
7	Bamboo clump	240	10	0	0	0
8	Plastic container	240	10	37 (15.4)	16.97	1.69
9	Metal drum	240	10	30 (12.5)	13.76	1.37
10	Plant pot	240	10	0	0	0
	TOTAL	2400		218 (9.08)		
	Indoor Containers					
1	Earthen pot	240	20	24 (10)	12.18	0.60
2	Cement tank	240	20	64 (26.6)	32.48	1.62
3	Plastic drum	240	20	53 (22.08)	26.90	1.34
4	Flower pot	240	20	10 (4.1)	5.07	0.25
5	Plastic bucket	240	20	46 (19.1)	23.35	1.16
	TOTAL	1200		197 (16.4)		

pots and minimum for a flower pots. The Breeding Preference Ratio (BPR) for all selected areas are presented in Tables 7-12. It was also noticed that the prevalence of *An. subpictus* was more in July to September and January to February in most of selected areas.

Discussion

Vector-borne infectious diseases are emerging or resurging as a result of changes in public health policy, demographic and societal changes, insecticide and drug resistance, a shift in emphasis from prevention to emergency response, and genetic changes in pathogens. Despite extensive research in the field of vaccine development, till now, no effective vaccine is available for mosquito vector-borne diseases. Therefore, the only way to avoid mosquito vector-borne diseases is to reduce mosquito density and humans-mosquito contact. Adult mosquitoes are difficult to control since they can fly relatively long distances and survive in a wide range of microhabitats. So it is crucial to check mosquito larval populations which have limited habitat and larval survey is the most widely used method for entomological surveillance. Accurately estimating site-specific mosquito larval abundance is central to vector control strategies. Larval abundance assay provides information related to bio-ecological aspects of mosquito species. This larval abundance assay is helpful to assess the impact of vector control measures, by comparing larval densities and breeding site occupation before and after the implementation of the intervention. For this, several indices have been used to measure the abundance of mosquito vectors for vector-borne disease transmission. Those indices related to measuring the immature populations are the House Index (HI), i.e., the percentage of a house infested with larvae or pupae and the Container Index (CI), i.e., the percentage of water-holding containers infested with larvae or pupae. *Anopheles subpictus* is a prevalent vector of malaria in Asia. It was previously reported in different states of India like Tamil Nadu (Paniker *et al.*, 1984), Madhya Pradesh (Kulkarni, 1983), Rajasthan (Tyagi and Yadav, 2001), Pondicherry (Panicker *et al.*, 1981), West

Bengal (Chatterjee and Chandra, 2000), Delhi (Sharma *et al.*, 1985), Chhattisgarh (Parmanand *et al.*, 2008). This species was also earlier reported in different districts of Odisha state like Angul, Khurda, Cuttack and Puri (Kumari *et al.*, 2013), Malkangiri (Sahu, 1998), Keonjhar (Sahu *et al.*, 2009), Nayagarh, Jagatsinghpur, Dhenkanal, Ganjam (Tripathy *et al.*, 2010). Vectorial capacity of *An. subpictus* was previously reported in different districts of Odisha including Ganjam (Tripathy *et al.*, 2010).

The Breeding Preference Ratio (BPR) was calculated as the ratio between IBS and IAC for each category. The Index of Available Containers (IAC) was estimated as the ratio of containers from each category divided by the total no of containers in houses containing at least one water container. Similarly, the Index of Contribution to Breeding Sites (ICBS) was estimated as the ratio of positive containers from each category divided by the total no. of positive containers in houses with positive containers (Flaibani *et al.*, 2020). The present study was undertaken in 6 different localities of the Ganjam district of Odisha state, India, to determine the breeding preferences of *An. subpictus* mosquitoes by conducting a larval survey in both outdoor as well as indoor containers and the Breeding Preference Ratio (BPR) was calculated. The results of the current study showed that *An. subpictus* preferred mostly cement tanks and earthen pots over other types of indoor as well as outdoor containers for oviposition. Previously many breeding sites for *An. subpictus* have been reported such as wastewater collections, lagoon water collections, and drains in some areas of Srilanka (Gunathilaka *et al.*, 2019), artificial ponds, paddy fields, canals, seepage, pools of the canal, wells, cemented tanks in Dhenkanal district (Panigrahi and Mahapatra, 2013). Further, based on the calculated BPR, it has been observed that *An. subpictus* preferred indoor sites for oviposition to outdoor sites. This could possibly due to the reason that, *An. subpictus* searches for the maximum availability of blood feeding from the inhabitants. The choice of a wide variety of breeding habitats near human dwellings makes this species a dominant vector over other anopheline species. It has been reported that Aedes mosquitoes preferred earthen pots both the indoors and outdoors and discarded tyres, plant pots and old vehicles/boats lying outdoors for laying their eggs. Discarded tyres and old vehicles/boats are found to be the best preferred breeding sites as the water remains undisturbed in them (Panigrahi *et al.*, 2013).

Anopheles subpictus was abundant during the monsoon period (Bansal and Singh, 1993) and captured in high numbers from May to July (117/501, 23.3 %), but prevalence continued later including a prominent peak between December and April (334/501, 66.7 %) in Goa (Kumar *et al.*, 2016). During month-wise container screening it was noticed that the prevalence of *An. subpictus* was more in July to September and January to February which might be due to variations in rainfall. Similar kind of results was also recorded by Dash *et al.*, (1998) where they found the peak density of *Culex quinquefasciatus* in January in Khurda district of Orissa. Dixit *et al.*, (2002) in Raipur, Chattisgarh recorded the prevalence of the species in all months of the year with high densities during February and March months and no significant relationship was observed between the variations in density of the vector and the variations in temperature and/or humidity in the study area. Similarly, Dhar *et al.*, (1968) in their study on *Culex quinquefasciatus* in Rajahmundry town of Andhra Pradesh, observed the prevalence of the adults in all months of the year with a definite upward trend in density which started from July-August in the monsoon period and reached the peak in November-December in the post-monsoon period. The prevalence of the indoor-resting population of *An. subpictus* was higher in the rainy season may be due to greater breeding facilities during the rainy season in the paddy fields, shallow ponds and also in temporary water collections (Chatterjee and Chandra, 2000).

Conclusion

The current study provides useful information on the mosquito population dynamics and breeding habitats of *An. subpictus* across urban and rural areas of Ganjam district of Odisha state. The results of the present study provide important information for improving larval source management operations for the control of malaria vectors and help to prevent the outbreak of future malaria epidemics particularly in these studied areas.

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